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14. ABSTRACT
This program is designed to explore the militarily-relevant medical applications of high-power, tunable infrared radiation available from unique accelerator-based light sources, the Mark III FEL, the monochromatic X-ray system, and a new Smith-Purcell THz FEL. As applications mature, we also develop cost-efficient, dedicated systems to expand the use of these applications beyond the FEL Center. During the last funding period, we have developed a comprehensive research program directed towards a thorough understanding of militarily-relevant laser medicine applications. These studies involve substantial collaborations with all of the other FEL centers: dermatology with the Wellman Laboratories, infection and cancer diagnosis with Stanford, bone and cartilage ablation with UC Irvine, neurosurgery with Duke. Since the Vanderbilt center has the only FEL that is coupled to an FDA-approved human operating rooms, all FEL clinical applications developed within the MFEL program are carried out at Vanderbilt. In particular, our researchers have concentrated on the development of three new surgical approaches using the FEL, 1) decrease of wound healing and scar minimization, 2) ophthalmological surgery in the back of the eye without removing the eye from the socket, and 3) laser-based non-contact nerve stimulation.

15. SUBJECT TERMS
Laser, free electron, FEL, infrared, tunable, wavelength, ablation, neurosurgery, dermatology, ophthalmology, radiation, monochromatic X-ray, Smith-Purcell, THz, terahertz, infection, cancer, bone, cartilage, wound healing, scar, human optic nerve sheath, nerve stimulation.

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Covering the performance period ending May 31, 2004

This program is designed to explore the applications of the high-power, tunable infrared radiation available from unique accelerator-based light sources, the Mark III FEL, the monochromatic X-ray system, and a new Smith-Purcell THz FEL. As applications mature, we also develop cost-efficient, dedicated systems to expand the use of these applications beyond the FEL Center.

During the previous funding period, we have developed a comprehensive research program directed towards a thorough understanding of militarily-relevant laser medicine applications. As an important part of the overall Medical Free Electron Laser program, these studies involve substantial collaborations with all of the other FEL centers: dermatology with the Wellman Laboratories, infection and cancer diagnosis with Stanford, bone and cartilage ablation with UC Irvine, neurosurgery with Duke. Since the Vanderbilt center has the only FEL that is coupled to an FDA-approved human operating rooms, all FEL clinical applications developed within the MFEL program have been carried out here. The research teams at Vanderbilt have continued the development of three new surgical approaches using the FEL, 1) decrease of wound healing and scar minimization, 2) ophthalmological surgery in the back of the eye without removing the eye from the socket, and 3) laser-based non-contact nerve stimulation.

Our work in wound healing has led to new heat conducting diamond templates that significantly reduce the amount of lateral thermal damage associated with laser surgery. These results not only apply to our FEL surgeries, but also significantly improve conventional cutaneous laser surgery. We are hopeful that on-going clinical trials with CO_2 lasers will confirm our results and lead to wide-spread use of our approach. We have also found the FEL to be superior to the CO_2 resurfacing laser and dermabrasion in scar revision in a nude mouse model, and we are currently developing this for human surgery by combining our FEL template with the air cooling approach developed at the Wellman Laboratories. During these studies, we have discovered that differences in a key skin protein, MMP-13, directly correlate with wound healing. By inhibiting MMP-13, we have been able to reduce wound healing time in animal models from 50 days to 20 days. This approach could have serious relevance to military injuries. Ophthalmology applications center around the coupling of the FEL an orbital endoscope that we have developed to reach the back of the eye. Currently all surgery behind the eye require the eye to be removed from the socket, which leads to increased risk of infection and extended convalescence time. The results in animal models were superb, and we are currently applying to the Vanderbilt IRB and FDA to pursue human studies with this device. We have successfully completed the human clinical studies of the FEL cutting of the optical nerve sheath, and this procedure will be the first FEL study performed with the new orbital endoscope.

We have recently discovered that that low intensity FEL light can stimulate nerve action potentials. This stimulation occurs at levels well below the ablation threshhold, which we have well characterized during other human neurosurgical applications (now completed). We are moving from our animal studies towards human clinical studies. We plan to test the efficacy of optical stimulation in motor nerve by stimulating the median nerve in a patient undergoing a carpal tunnel release operation. Electrical stimulation will be used for comparison. This laser stimulation approach has tremendous potential for rehabilitation of all nerve damage, which is a major concern after traumatic injuries, such as combat casualties

In addition to surgical applications, we have also discovered and developed novel methods for detection of biological or chemical constituents. These techniques are directly applicable to biological or chemical weapons screens, or to non-invasive imaging of packages and luggage. These techniques are based on the IR-MALDI approach that has been developed over the last 5 years and our new Smith-Purcell THz FEL. IR-MALDI offers the potential of detecting biological molecules biopsied or cultured tissue with sensitivity that is 100 fold better than conventional MALDI. We are developing this approach for detection of pathogens in animal models before any symptoms are visible from the animal; such an approach will allow detection and classification of new diseases or a bioterrorism attack. THz imaging is less well developed, but it also offers the potential of specific chemical imaging. Every chemical has a unique spectroscopic signature in the THz region (much like many chemicals are colored differently in the visible light region), and this unique signature can be used to identify those chemicals. In addition, THz can be used to look through clothing and luggage in search of contraband such as weapons, drugs, or explosives. Our new compact Smith-Purcell FEL is the only source that provides sufficient THz intensity for these applications.

One of the strengths of the Vanderbilt FEL Center is its multi-disciplinary approach. Since this is different than most scientists' approach to research, we spend considerable effort in education and outreach. In addition to undergraduate and graduate level courses, we do community outreach. Every summer, we participate in two Summer Science camps for high school students, and in the summer high school science teachers' workshop. The FEL also forms one of the central educational resources for the two day Girls and Science program, which is aimed towards junior high school girls, who have historically demonstrated an interest in science, but for various reasons, have not pursued that interest.

FEL Operations

Bill Gabella, Ph.D., Bibo Feng, Ph.D., John Kozub, Ph.D., Marcus Mendenhall, Ph.D.

One of the most important tasks for the Vanderbilt FEL Center is reliable delivery of the FEL beam. During 2001, the Vanderbilt FEL delivered 2,016 hours of beam time to users, plus 373 more hours used for beam diagnostics and optimization by the Center staff, and in 2002, we delivered 2,307 hours of FEL beam time, with an additional 341 diagnostic hours. In 2003, we delivered 2,118 hours to users and an additional 487 diagnostic hours. In addition, the Center staff is responsible for running and maintaining the tunable optical parametric generator laser system, and the tunable monochromatic X-ray source. These responsibilities also include aiding researchers in the setup and execution of experiments.

Proteomics and Imaging

Richard Haglund, Ph.D., E. Duco Jansen, Ph.D., David W. Piston, Ph.D.

Some of these projects are solely supported by the MFEL program, and others are jointly supported by our National Cancer Institute *In Vivo* Imaging Center grant. This group of investigators also interfaces with the 6.45 mechanism project.

IR-MALDI for Proteomic Applications

We have recently shown that angiotensin II and bovine insulin may be successfully desorbed and ionized from a frozen polyacrylamide gel using the FEL at wavelengths of 5.7 – 6.3 μm without

treating the gel with any additional matrix. Results from this work, shown in Figure 1, indicate that the normalized ion signal is wavelength dependent in this OH-bend absorption region; no signal was observed at 5.5 μm, regardless of the deposited energy. The adaptation of the FEL to a state-of-the-art mass spectrometer (currently under way) will allow us to evaluate the applicability of this technique to higher molecular weight proteins.

We have also shown that angiotensin II may be successfully desorbed and ionized from a frozen aqueous solution using the FEL at wavelengths around 5.9 μm [Baltz-Knorr 2002b]. Figure 2 represents recent work which shows that, similar to the results for gels, the normalized ion signal is also wavelength dependent. However, unlike gels, some signal was observed at 5.5 μm at high fluences. Second, the doubly charged peptide is often observed from ice but not from gel. Third, we regularly observe the formation of water clusters from ice (low-mass region of Figure 2), but never from gels.

We have begun to investigate the conditions necessary for the formation of water clusters, assuming that these clusters are relevant to the analyte ionization process. Generally, we observe that analyte ion signal appears after three or four laser shots, which is consistent with removal of contaminants and frost that condenses on the surface during sample preparation and manipulation, although sometimes no signal is observed for the first ten or twenty shots. The analyte signal typically appears for several shots, and then may diminish, analogous to other "first shot" experiments [Cohen 1997]. Water clusters appear to "grow in;" the average size of the observed water cluster decreases with successive laser shots. Eventually, there are almost no clusters (and usually no analyte) observed. Our initial conclusion was that the spot had been depleted of usable sample. Since then, however, we have found that with more careful handling and "bottom up" freezing of the sample, we often are able to generate analyte signals from the same spot even after many dozens of shots. Although we may observe excellent single-shot spectra, we only observe these for *ca.* 1/3 of the shots; while this is an improvement over previous attempts using IR-MALDI or LDI on ice, it is not yet comparable to the shot-to-shot consistency of a well-prepared crystalline matrix sample (albeit UV-MALDI still involves a great deal of "art" in sample preparation).

Effects of pulse structure on tissue ablation by 6.45 μm

We have developed a method to stretch the FEL micropulses so that each macropulse can be a string of 1 psec pulses or longer pulses (all the way to the micropulse spacing (350 psec). We are continuing the analysis of acoustic transients, ablation thresholds, and tissue removal characteristics with these different pulse widths. This data will be synthesized to arrive at a conclusion regarding the role of pulse structure on the FEL ablation process. We are also continuing our attempts to generate experimental comparisons with other 6.45 mm laser sources. In collaboration with developed by Dr. Soldatov, at the University of Tomsk, Russia, we have built and are currently testing a Strontium vapor laser (1 nec pulses, 1 kHz, and 100 mW average power). Both the peak and average powers available from this laser are comparable to those obtained from the FEL at 6.45 μm. Unless there is something special about the 1 psec pulse with a 350 psec spacing, the Strontium vapor laser should be an excellent system for tissue ablation. The second non-FEL source we are investigating is a high pulse energy OPO based on an Er:YAG pump (developed by Dr. Ramesh Shori, UCLA). The OPO system will be available for us to use at the UCLA campus.

Very high resolution near-field chemical imaging using an infrared free electron laser

We have built a reflection SNOM system, where the entire sample is illuminated by the IR light, and the reflected signal is collected with a narrow fiber tip aperture ($<\lambda$) placed at a distance smaller than λ from the sample surface. The use of SNOM allows us to greatly surpass the diffraction limited resolution, and easily visualize sub-cellular structures. The full potential of IR-SNOM, however, is not exploited without using a "spectroscopic" approach, i.e., taking images at different wavelengths to detect specific vibrational modes characteristic of chemical constituents The power of such a spectroscopic approach in IR-SNOM has been demonstrated in semiconductors, polymers, chemical, and biological systems [Cricenti et al., 1998; Cricenti et al., 2003]. Here, we describe our results using this approach to image biological systems, including live cells.

Surgical Photonics Applications

Cutaneous Surgery
Darrel Ellis M.D., Jeff Davidson, Ph.D., Duco Jansen, Ph.D., Lillian Nanney, Ph.D., George Stricklin, M.D., Ph.D.

Our goals have been to 1) Reduce cutaneous wound healing time and minimize the resulting scar with the FEL, 2) Develop the capacity to use the FEL as an incisional and ablational device for human cutaneous surgery and 3) Generalize findings we have made in our experiments with the FEL for use with other laser systems. Our latest work has been *in vitro* with excess human breast tissue, and *in vivo* with rats. We previously found reduced thermal damage with the use of heat-conducting templates, initially using copper and aluminum templates (recent publications 1,2). We measured the FEL beam diameter to be 636 ± 100 µm, indicating the aperture in the template needs to be approximately 700 µm (1). We have expanded this and confirmed the similar utility of sapphire templates in a paper just submitted entitled "Wound Healing of 6.45 µm FEL Skin Incisions with Heat-Conducting Templates" (see manuscript). A clear template is important for use in cutaneous surgery, where the operating field needs to be visualized by the surgeon. The paper just submitted showed trends for increased early tensile strengths in wounds treated with the FEL and heat conducting templates vs. the FEL alone, as well as a trend for a more mature early wound healing response in rats when the templates were used. We also have had success in translating the use of heat conducting templates with other laser systems. In the paper accepted for publication in Lasers in Surgery and Medicine entitled "Reduction in Lateral Thermal Damage Using Heat-Conducting Templates: A Comparison of Continuous Wave and Pulsed CO_2 Lasers" (see manuscript), we found that the heat conducting templates significantly reduced the amount of lateral thermal damage *in vitro* with both a 0.2 sec shuttered continuous wave and a 5 µsec short pulsed CO_2 laser system.. The templates allowed the long pulsed CO_2 laser to produce much less thermal damage, and compare favorably to the short pulsed CO_2 laser. This has implications for use in possibly replacing scalpels for cutaneous surgery. We plan to pursue this by investigating the use of a better heat conductor (diamond) with the long pulsed CO_2 laser. A grant from the American Society for Laser Surgery and Medicine has been applied for and will be conducted by a former FEL fellow who is now a resident in Dermatology at Vanderbilt University (Jason Robbins, M.D.) for this project under my supervision (Darrel L. Ellis, M.D.). Thus the FEL fellowship has sparked continuous interest in laser surgery applications.

We have also found the FEL to be superior to the CO_2 resurfacing laser and dermabrasion in scar revision in a nude mouse model (3), and plan to develop this into an application for human surgery. We are therefore working to develop a suitable delivery system for the FEL for human dermatologic surgery. To reduce thermal injury from the FEL for human cutaneous surgery, we are testing two methods. The template method has proven to be experimentally sound, but requires considerable engineering to be practical with the FEL. We are planning to develop a diamond thermal conducting template hand piece, which will allow excellent thermal conductivity and visibility for the surgeon, and should be applicable to many laser systems. This will be developed with two diamond windows forming a central slit for the FEL beam, which are available through Harris International. We are also testing the Zimmer Cryo 5 air cooling system to see if it is possible to limit thermal damage with air cooling, thus decreasing the engineering requirements for the FEL hand piece. These experiments in reducing thermal tissue damage continue to be conducted with Dr. Lou Reinisch, who travels from New Zealand at regular intervals to collaborate on the experimental design and execution. The histologic data from the initial experiments on the Zimmer unit are not available yet. Assuming the data are promising, we plan to use the air cooled unit in an animal model. Either rats or piglets will be used. Piglets more closely approximate human skin, but are also more expensive to use. Therefore the initial experiments may be conducted in rats. Once a proper system for reduction of thermal damage with the diamond template or air cooling has been shown to be effective *in vitro* in human skin and *in vivo* in animals with the FEL, we plan to advance to the human application of the FEL for incisional surgery (excising cutaneous lesions) and ablational surgery (scar revision).

Wound Healing
Jeffrey M. Davidson, Ph.D., E. Duco Jansen, Ph.D.; Lillian Nanney, Ph.D., NanJun Wu, Ph.D.

The main goal of this phase of the program has been to understand the molecular basis for differences in healing between laser and conventional surgery. These studies began with the observation that the Vanderbilt FEL produced reduced collateral damage in the skin at 6.1-6.45μ. Initial studies with the adult rat showed that FEL-incised wounds in fact healed better (more rapid restoration of tensile strength) than surgical wounds or wounds with conventional IR lasers.

We have developed three novel, noninvasive transgenic animal models for investigating laser tissue interactions in mice: each of these use a unique gene promoter that drives the expression of two marker genes – firefly luciferase and green fluorescent protein. The combination of the two expressed genes allows one to visualize *in vivo* patterns and dynamics of gene expression during the course of cutaneous repair after laser or conventional surgery. The three genes evaluated thus far are collagen (type I), collagenase (MMP-13 in the mouse), and elastin. Each of these matrix molecules plays a critical role in tissue repair: collagen provides the major substrate for cell traction, collagenase is the rate-limiting enzyme for collagen remodeling, and elastin is an important determinant of skin resiliency and scar quality. Accomplishments under the present funding period include publications describing the development of the MMP-13 mouse and a study comparing patterns of gene expression in FEL versus scalpel wounds in mouse skin. An important finding was the biphasic pattern of collagenase expression. A spin-off from this project is the development of a new arthritis model.

One important approach to differentiating the effects of FEL surgery from other modalities is the analysis of gene expression. Our previous strategy has been driven by the

hypothesis that certain gene products are selectively affected, but this is strongly biased by the availability of reagents and techniques. Microarray analysis offers the possibility to screen broadly for multiple genes that are differentially expressed after laser or surgical injury to tissues. We utilized the Vanderbilt Microarray Core facility to perform a preliminary screen of RNA populations from FEL wounds produced at 6.1μm with those produced by scalpel at 1 day after surgery. This approach identified MRP-8 expression as a prominent difference, which was then quantified by Northern blot analysis of RNA from these wounds and localized to granulation tissue by nonradioactive *in situ* hybridization. MRP-8 is often expressed by inflammatory cells, and gene therapy with MRP-8 can accelerate wound healing.

Ophthalmology Applications
Karen Joos, MD, Ph.D., Jin Shen, Ph.D., Louise Mawn, M.D.

We have successfully utilized the FEL in several experimental ophthalmic surgical procedures over the past 8 years and have advanced to using the FEL in experimental human ocular surgery. The initial key advancement was identifying laser-tissue interactions at specific wavelengths with the least collateral damage at Amide I (6.0 μm) for cornea, no collateral damage at Amide II (6.45 μm) for optic nerve, and no collateral damage at 2.94, 6.0, 6.1, and 6.45 μm for retina [Joos et al., 1996]. We successfully developed sterile prototype glass-hollow waveguide surgical probes, which we continue to improve for delivery of infrared energy [Shen et al., 2001]. In rabbits, the FEL at 6.45 μm delivered through the waveguides within the aqueous environment of the eye easily transected vitreous bands that were formed by injecting fibroblasts intravitreally to produce this model of trauma [Shen et al., 1999]. The FEL energy needed for effective band transection was only 2-3 mJ. Addition of low aspiration would be desirable for improving band transection efficiency. Optic nerve sheath fenestration is a surgical procedure to relieve increased pressure around the optic nerve in idiopathic intracranial hypertension by cutting a window in the dural covering. Surgery using the FEL probe at 6.45 μm was found to be technically superior to the knife in performing this procedure in rabbits [Joos et al, 1998; 2000] and monkeys [Casagrande et al., 1999; Joos et al., 2003]. Immunohistochemistry demonstrated equal glial cellular reactions between the 2 methods both acutely and 1 month after healing, and showed that the FEL energy effectively cut the sheath without damaging the underlying optic nerve. A series comparing the FEL with the scissors in human optic nerve sheath fenestration in blind eyes prior to enucleation is nearing completion. The initial FEL case suggested surgical ease [Joos et al., 2002].

The FEL has now been combined with an endoscope both intraocularly to successfully perform endoscopic goniotomy in rabbits with congenital glaucoma [Sun et al., 2000], and extraocularly to incise the optic nerve sheath [Mawn et al., 2003]. However, the extraocular instrument and procedure is currently cumbersome and time-consuming, and requires refinement before it becomes clinically useful. The aim of this proposal is to refine and improve the FEL coupled endoscope to be operator-efficient and useful for FEL-based ophthalmic surgery. Success would significantly advance minimally invasive endoscopic FEL surgery as a potential human surgical technique.An exciting development in the FEL eye surgey has come from the attainment of NIH funding for this project. This funding will allow us to develop a usable endoscope for the optical nerve fenestration surgery (as well as other surgeries behind the eye) in a way that does not require removal of the eyeball before the surgery can proceed. The details of this grant are: Development of an Orbital Endoscope for Neuroprotection, Louise

Mawn PI, Karen Joos co-investigator, National Institutes of Health, National Eye Institute, RO3EY013800 August 1, 2001 – July 31, 2004, $300,000 total.

Neurosurgical Applications – Nerve Stimulation
Pete Konrad, MD, Jeff Albea, MD, Anita Mahadevan-Jansen, Ph.D.

Over the past year a total of eighteen wavelengths were studied to determine nerve stimulation and ablation thresholds. The wavelength range was 2.8-6.2um advancing in .2um increments. From this initial data, four wavelengths were chosen (4μm, 4.4μm, 5μm, 5.4μm) for histologic study. Stimulation thresholds were found to be 4μm = 0.557J/cm^2, 4.4μm = 0.395 J/cm^2, 5μm = 0.301 J/cm^2, 5.4μm = 0.479 J/cm^2, and ablation thresholds were found to be 4μm = 1.96 J/cm^2, 4.4μm = 2.29 J/cm^2, 5μm = 2.41 J/cm^2, 5.4μm = 1.9 J/cm^2. Currently, we have submitted 27 nerves for histology. Once we review the nerve tissue slides for adequacy of the preparations they will be sent to a pathologist specializing in optically damaged tissue for a formal reading. A labeling system was devised so that the interpreting pathologist will be blinded as to the wavelength and energy used on a particular nerve.

We have now shown that pulsed laser energy can effectively mimic the effects of electrical stimulation on peripheral nerves in frogs and rats. This novel form of neural stimulation has many potential advantages, including precision of the tissue being stimulated, lack of an electrical stimulation artifact, and lack of an electrode-tissue interface when the tissue is being stimulated. Presently we are completing safety studies to determine the energy differences between minimally effective stimulation of peripheral nerve tissue and minimum energy required to produce histological damage to the tissue, and moving this application to clinical use (see section 4.d below). However, the phenomenon of the ability of pulsed laser energy to cause action potentials in peripheral nerves is proven in both mammalian and amphibian models. We do yet not know if pulsed laser energy can cause action potentials in neuronal cell bodies. An excellent model we have used to study *in vitro* neuronal cell and network activity is the rat, thalamocortical (TC) slice preparation. This preparation preserves a three neuron network between cortical and thalamic neurons that reproduces action potential activity similar to that seen with *in vivo* animal studies and humans. This model will allow basic investigation into the fundamental neurophysiological parameters that have been characterized for electrical stimulation. In fact, these fundamental properties can be directly compared with our apparatus that allows a simultaneous comparison of laser versus electrical stimulation responses.

Outreach and education

One of the strengths of the FEL Center is its multi-disciplinary approach. Since this is different than most scientists' approach to research, we spend considerable effort in education and outreach. In addition to a new graduate level course on Physical Measurement of Biological Systems, we do quite a bit of community outreach. Every summer, we participate in two Summer Science camps (run by Prof. Joe Hamilton in Physics), and we have done demonstrations and hands-on teaching for the summer high school science teachers workshop. The FEL also forms one of the central educational resources for the two day Girls and Science program (run by Prof. Virginia Shepherd and Charlie Brau) several times each year. This second program is aimed towards junior high school girls, who have historically demonstrated an

interest in science, but for various reasons, have not pursued that interest. This year, the FEL has also hosted the Vanderbilt University Retirement learning class, performed alumni college classes and tours for the yearly reunion, and taught the scientific research explorers troop (Boy Scouts of America).

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